

***In Vitro* Amplification Techniques for the Detection of Nucleic Acids: New Tools for the Diagnostic Laboratory**

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The acceptance of nucleic acid probes as diagnostic tools for the clinical laboratory has been hampered by a number of factors, including laborious techniques and limited sensitivity. The focus of this review is on the recent development of amplification techniques to enhance the signal generated by nucleic acid-based detection systems. Three general areas are discussed: (1) amplification of target sequences using the polymerase chain reaction or the transcript amplification system, (2) amplification of the probe sequences using Q β replicase, and (3) amplification of probe-generated signals with compound or "Christmas tree" probes. The hope of these new technologies is to simplify yet improve on the sensitivity of nucleic acid-based tests to enable them to attain a more prominent place in the diagnostic repertoire of the clinical laboratory.

INTRODUCTION

The application of the techniques of modern molecular biology to the diagnosis of human disease has recently made significant advances. Some of the most successful applications have been in clinical conditions where classical biochemical markers are either unknown or are inaccessible; under these circumstances, nucleic acid analysis may be the only means of providing diagnostically relevant information [1]. Examples include restriction fragment length polymorphism (RFLP) analysis in the diagnosis of Huntington's chorea trait in affected families [2,3], cystic fibrosis heterozygosity in genetic screening programs [4], and gene rearrangements in lymphomas and leukemias [5]. Other efforts have focused on the diagnosis of infectious agents; the agents chosen for study have typically been those whose identification and characterization are hampered by the lack of a suitable system for *in vitro* cultivation or a paucity of identifying features [7,9-11].

Despite such advances, however, the acceptance of nucleic acid probes as diagnostic tools in the clinical laboratory has been slow to develop. This delay has generally been ascribed to the difficulties in conveying a basic research tool to the clinical laboratory. If maximum sensitivity is sought, radiolabeling of such probes with potentially hazardous radioisotopes such as ³²P is a requirement. The relatively short half-life of ³²P-labeled probes dictates that the probes be resynthesized on a regular basis, thus increasing radioisotope exposure and intensifying the need for stringent batch-to-batch

Abbreviations: cDNA: complementary DNA CMV: cytomegalovirus HIV-1: human immunodeficiency virus type 1 LTR: long terminal repeat PCR: polymerase chain reaction RFLP: restriction fragment length polymorphism TAS: transcript amplification system TCID₅₀: 50 percent tissue-culture infectious doses

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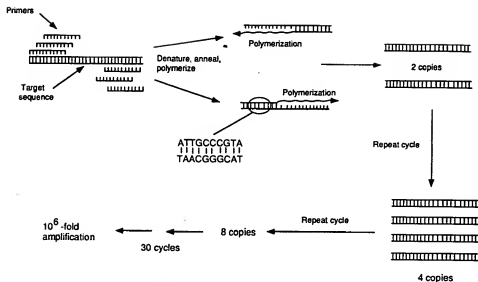


FIG. 1. The polymerase chain reaction. Each cycle of PCR consists of three steps: (1) a denaturing step, in which the target DNA is incubated at high temperature so that the target strands are separated ("melted") and thus made accessible to the excess primers present in the reaction buffer; (2) an annealing step, in which the reaction mixture is cooled to allow the primers to anneal to their complementary sequences on the target sequences; and (3) an extension reaction, usually carried out at an intermediate temperature, in which the primers are extended on the DNA template by a DNA polymerase. The extension of each strand results in the synthesis of a primer binding site for the next round of replication; as a result of the fixed distance between binding sites, the predominant amplification product is of discrete length—that of the distance between the outermost ends of the primers as they are situated on the target DNA. Each time a cycle is completed, there is a theoretical doubling of the target sequence.

probe specific for the amplified region. Alternatively, dot-blot hybridization can be performed with no loss of sensitivity or specificity [19].

The primers are oriented in such a manner that DNA synthesis proceeds toward the annealing site for the primer on the opposite strand; herein lies the process by which PCR gains much of its specificity. While the probability is quite high that a given primer will anneal nonspecifically to multiple sites in the target material, the probability that a second primer will reproducibly (but nonspecifically) anneal to the opposite strand at a precise position and in close proximity to the first is very low. In general, the specifically amplified target sequence is the predominant amplification product and is easily identified by its precisely defined length; nonspecific amplification products tend to be heterogeneous in size, and they do not usually become the predominant product.

Two recent technical innovations have simultaneously simplified and greatly increased the power of PCR. The discovery that the thermostable DNA polymerase (*Taq* polymerase) [47], isolated from the thermophilic bacterium *Thermus aquaticus*, is able to withstand repeated cycles of heating to 95°C has made it possible to carry out PCR without reopening tubes and adding fresh polymerase after each denaturing step. *Taq* polymerase is added once at the beginning of the reaction, thus greatly simplifying tube handling. Furthermore, the ability to perform annealing and extension reactions at a higher temperature significantly reduces nonspecific amplification; the higher temperatures are unfavorable for the formation of imperfectly base-paired complexes between primer and target. A second innovation is the development of the program-